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Shell Microstructure and Color Changes in Stressed *Corbicula fluminea* (Bivalvia: Corbiculidae)

by

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Abstract. Specimens of *Corbicula fluminea* from Mississippi show altered internal shell color and microstructure when maintained under stressful conditions. Clams under laboratory conditions that "threaten" life-sustaining functions, as well as field collections of moribund animals, show conversion of typically purple highlighted internal shell to pure white, and conversion of a surficial complex crossed-lamellar microstructure to a form reminiscent of complex crossed-acicular. These changes possibly result from a shift in metabolic activities away from energy expenditures normally allocated to shell production and towards life-sustaining functions. Alternatively, the modified shell can be a reflection of internal shell dissolution under similar conditions, although dissolution studies reveal a different form of shell erosion. Additionally, crossed-acicular microstructures, at least as they appear in exposed shell layers, could be an environmentally induced product and should not be construed as a product of "normal" biomineralization processes.

INTRODUCTION

The question of environmentally induced changes in corbiculid shell form and structure has become more than an esoteric exercise in taxonomy. The interest coincides with the rapid invasion of *Corbicula* throughout North America (McMahon, 1982; Counts, 1986) and the incessant debate over the number of species occurring in North America. Prashad (1929) and Sinclair & Isom (1963) discussed variability in shell characters in the genus *Corbicula,* but Morton (1986) and Britton & Morton (1979) claimed that taxonomists have not distinguished significant differences in shell form within the genus but rather have overemphasized "slight or moderate variability in conservative shell characteristics." During the First International *Corbicula* Symposium, Britton & Morton (1979) suggested that a single species occurred in North America. Hillis & Patton (1979) and, more recently, McLeod (1986) presented electrophoretic evidence for two species of North American *Corbicula* (*i.e., C. fluminea* (Müller) and inconclusively *C. fluminalis* (Müller): the so-called white and purple forms respectively). Hillis & Patton (1979) also presented morphometric differences in shell form between their electrophoretically separated forms. One form, with a deeply purple internal shell, also has more shell annuli than the "white" form. Prezant & Tan Tiu (1985) found few significant microstructural shell differences between the two color "forms" but did note a slight (ca. 0.15%) difference in total shell organics, with the purple form having slightly more. The previous study did not include an examination of shell recessed beneath the peripherally undertucked periostracum. When this in-

ternal edge was examined (Prezant & Tan Tiu, 1986) a unique shell microstructure (spiral crossed-lamellar) was found in a large number of white forms of *Corbicula* (*i.e.*, *C. fluminea*) but has yet to be found in the purple form.

Since populations of *Corbicula* are found in North American lotic and lentic habitats (Britton, 1982) in various substrata over many ecologic provinces (McMahon, 1982), the bivalve may be suspected of being morphologically plastic. This paper reports variations in intraspecific internal shell color and microstructure in "white" *Corbicula* (*i.e.*, *C. fluminea*) under stressful environmental conditions. We have found that internal shell microstructures of *C. fluminea* are variable and can reflect environmental or physiological conditions. We further suggest that a crossed-acicular microstructure, as it appears in exposed shell layers, is an environmentally induced modification of a crossed-lamellar microstructure and should not be construed as a specific calcified microstructure.

MATERIALS AND METHODS

Living specimens of *Corbicula fluminea* were collected from sand and gravel bars in Tallahala Creek, Perry County, and from sand deposits within eroded rock "pools" in Strong River at D'Lo, Simpson County, Mississippi, U.S.A. All clams collected were of Hillis & Patton's (1979) white form (based on dimensions, shell shape, and annulus counts) but most exhibited a purple highlighted shell internally (though not the homogeneous, deep purple pigmentation of the "purple" or dark form).

Live animals, for baseline analyses, were immediately shucked upon collection or upon return to the laboratory. Valves were prepared for scanning electron microscopy as follows. Valves were dehydrated in absolute ethanol for 5–9 days prior to critical point drying in a Denton DCP-1 critical point drying unit. Liquid carbon dioxide was used as a direct transfer agent. Some specimens were fractured prior to drying. All specimens were mounted on aluminum stubs using silver paint and coated with a thin layer of gold. Specimens were examined at the University of Southern Mississippi in an AMR 1000A scanning electron microscope (SEM) at an accelerating voltage of 30 kV.

Initial experiments involved manipulating temperature and "food" regime using aged tap water at ambient and warm temperatures. (Food is placed in quotation marks to reflect the difficulties in getting active growth [*i.e.*, comparable to field growth] in the laboratory. While easy to maintain in apparent good health and with slight growth, large size and weight gains over long periods have yet to be reported for laboratory populations of *Corbicula fluminea*; in fact minimal and even degrowth are more common.) Four separate 38-L aquaria, with gentle outside filtration (with removable activated charcoal filters), were used to each hold six small (L = 6–12 mm) (L = maximum anteroposterior shell length) *C. fluminea* collected from Tallahala Creek in September 1982. Test animals were held in the individual test aquaria at 23°C (ambient field temperature at time of collection) and two aquaria were gradually brought up to 31°C over a 4-day period. Individual aquaria were then held at 23°C without "food," 23°C with "food," 31°C without "food," and 31°C with "food." The feeding regime followed a 3-day cycle. A pure concentrate of liquified (well-blended) spinach leaves was added to the "fed" aquaria in doses of 25 mL every 3 days. Just prior to feeding, outside filtration packs in all aquaria were removed and not replaced for 24 h. Aquaria were held at 12:12 light:dark cycles using 40 W fluorescent tubes located 10 cm above the water surface. A sudden power surge brought the heated, "fed" aquarium to 37°C 3 weeks into the experiment and resulted in strong physiological stress of specimens in that tank within the next week's time. Clams in the afflicted tank were carefully monitored and removed immediately upon observation of death or impending death (*i.e.*, gaping and nonresponsive). Experiments in all aquaria were terminated 4 weeks after initiation and valves prepared for SEM studies as noted above.

24-Hour Adduction

To examine *Corbicula* shell for internal shell erosion that could have co-occurred with stress induced during the previous experiment, studies were conducted to induce internal shell dissolution as might occur during anaerobiosis (Table 1).

At 1045 h on 10 November 1984, 250 clams (L = 5–15 mm) were collected from the Strong River. At the time of collection, air temperature was 20°C, water temperature was 18°C, pH 5.85, and conductivity was 62 mhos/cm. The water in the Strong River at D'Lo was running faster and higher than usual because of recent rains. Ten clams were sacrificed at 1130 h as baseline specimens. All other specimens were clamped shut (forced adduction) in the field and returned to the lab (ambient temperature = 19°C) where 10 specimens were sampled every hour for the next 25 h (the 0530 h sampling was missed so the study ran an additional hour to allow for 24 samples plus baseline). At sampling, the specimens were shucked, valves were prepared for SEM, and soft parts were preserved in Hollande-Bouine's fixative for future analysis.

7-Day Adduction

Clams (L = 5–15 mm) were collected from the Strong River on 10 November 1984 and held overnight in the laboratory in Strong River water at 18°C in a recirculating 19-L aquarium. Ten clams were sampled as a baseline and 70 others were force adducted using clothes pins and suspended in the aquarium. Each day for the next 7 days 10 additional clams were sampled. Specimens were prepared for SEM and soft parts fixed in Holland-Bouine's fixative for future analysis.

To explore the possibility that during dissolution calcium carbonate is preferentially lost leaving a remnant

Table 1

Sizes (averages) and results of 24-h *Corbicula fluminea* shell dissolution study. Experiment begun on 10 November and ended on 11 November 1984. (SD = standard deviation; *n* = number of individuals from sample examined by scanning electron microscopy.) Microstructural conditions indicated are from various regions of internal shell as indicated by code (A = under periostracum; B = just dorsal to periostracum; C = dorsal to B and inclusive of pallial line; D = just dorsal to pallial line; E and F = just dorsal to D; G = at level of ventral edge of adductor scars; H = at level of dorsal edge of adductor scars/lower edge of retractor scars; I = umbonal region). Only significant changes are noted.

Time	Length ± SD (mm)	Heigth ± SD (mm)	*n*	Microstruture condition
1130	13.9 ± 2.45	11.5 ± 1.93	9	Baseline condition.
1230	11.7 ± 1.06	10.1 ± 0.26	3	
1330	11.9 ± 1.38	10.2 ± 1.32	3	
1430	11.3 ± 0.91	9.4 ± 1.07	3	A–B: scattered pits. C: irregular pits; some fragmentation. D–F: narrowed lamels of irregular width. G–H: perforate blocks.
1530	11.7 ± 0.38	9.9 ± 0.23	3	G–H: cross-acicular pattern. I: smooth, porous.
1630	10.4 ± 1.30	8.6 ± 1.28	3	A–B: porous, some lamels protrude. C–F: lath fragments present, rare.
1730	11.3 ± 0.76	9.6 ± 0.83	3	A–B: lath fragments. C: highly porous.
1830	10.3 ± 1.30	9.2 ± 1.64	3	A–B: irregular laths, granular surface; porous. G–H: sharp-edged lamels, alveolar-like pattern mixed with smooth blocks. I: exposed laths with sharp, irregular surfaces.
1930	10.8 ± 0.80	9.3 ± 0.83	3	G–I: alveolar pattern dominates.
2030	9.0 ± 1.16	8.4 ± 0.86	3	
2130	11.6 ± 0.87	10.0 ± 0.95	3	
2230	10.6 ± 0.15	8.8 ± 0.00	3	A–B: irregular surface, porous, fragments, granules.
2330	11.0 ± 1.88	9.2 ± 0.93	3	C: smooth surface with scattered fragments, some granulate. G–H: alveolar, porous, blocky. I: alveolar rare.
0030	11.1 ± 0.20	8.8 ± 0.58	3	I: no alveolar or rare.
0130	10.4 ± 0.94	8.8 ± 1.14	3	
0230	11.0 ± 0.60	9.3 ± 0.53	3	G–H: alveolar with thick strands, blocks. I: deep alveolar structure.
0330	9.8 ± 3.00	8.1 ± 0.51	3	G–H: alveolar, lamels not readily discernible.
0430	11.5 ± 0.45	9.7 ± 0.50	3	D–F: lamels vague, short, sharp tips.
0630	11.9 ± 0.98	10.0 ± 1.05	3	
0730	10.7 ± 0.38	9.1 ± 0.45	3	
0830	11.1 ± 1.30	9.3 ± 0.98	3	
0930	10.3 ± 0.77	8.7 ± 1.10	3	
1030	11.1 ± 1.55	9.6 ± 1.31	3	I: no alveolar structures, smooth.
1130	10.9 ± 0.90	9.2 ± 0.88	3	I: heavy alveolar structures with deep pits.
1230	11.4 ± 0.56	9.5 ± 0.68	3	

organic matrix behind, a series of three recently shucked bivalve shells (L = 18.0–28.8 mm; Table 2) (sampled 5 June 1985) were subjected to a 30-sec treatment in 50% hydrochloric acid followed by a distilled water rinse and preparation for SEM.

Another series of three shucked valves (from the 7-day dissolution study), with valves showing reticulated structures, were subjected to a 5-min treatment in 5.25% sodium hypochlorite (commercial Clorox®) followed by distilled water rinses and preparation for SEM.

RESULTS

After a 3-week period, a sudden electrical power surge brought the 31°C "fed" aquarium up to 37°C. This resulted in physiologically stressed clams in this tank within the next 7 days. All clams that remained alive in each of the other tanks were sacrificed at the end of the 7th day in the 4th week. All clams in the ambient tanks, fed and nonfed, maintained a purple-brown highlighted internal shell coloration with a characteristic internal complex crossed-lamellar shell (*i.e.*, internal to pallial line) (Figure 1). Five of the six clams maintained through death or impending death in the hot, "fed" tank produced white internal shell surfaces with crossed-acicular patterns replacing the internal complex crossed-lamellar microstructure (Figure 2). A single clam in the warm, nonfed tank produced a white internal shell with "crossed-acicular" patterns, but all others had "normal" pigmentation and microstructure.

Table 2

Dates and sizes from 7-day dissolution study of *Corbicula fluminea*. Three specimens from each daily sample were examined via scanning electron microscopy. Year = 1984; measurements in mm.

Date	Length ± 1 SD	Width ± 1 SD	Height ± 1 SD
11 November	12.4 ± 2.55	7.8 ± 1.93	9.8 ± 1.36
12 November	11.9 ± 1.62	7.0 ± 0.87	10.1 ± 1.52
13 November	11.9 ± 1.72	7.5 ± 0.67	9.6 ± 1.35
14 November	11.6 ± 1.27	7.1 ± 0.85	8.7 ± 2.20
15 November	11.4 ± 2.34	8.0 ± 2.75	9.5 ± 2.86
16 November	11.5 ± 3.16	6.9 ± 1.99	9.8 ± 1.57
17 November	11.6 ± 1.72	7.0 ± 1.09	10.1 ± 2.57
18 November	12.3 ± 2.69	7.7 ± 2.12	9.4 ± 1.46
19 November*	11.7 ± 1.81	6.9 ± 1.29	

* Dead upon opening at termination of experiment.

Forced Adduction

24-h study (see Table 1): Internal to the pallial line after 16 h of forced adduction, obvious changes in shell microstructure occurred. These changes included increased porosity of organic layers in cross-lamellar structures (Figure 3), disruption of regular lamel tips (Figure 4), and finally a breakdown of organized lath orientation (Figures 5, 6). In some cases this type of dissolution or disruption of internal shell surface microstructure resembles a crossed-acicular pattern.

7-day study: During the first 5 days of forced adduction there was no mortality. All 10 clams sampled daily were alive up to day 6. On day 6, all 10 clams sampled were dead and on day 7, only 2 of 10 sampled were alive.

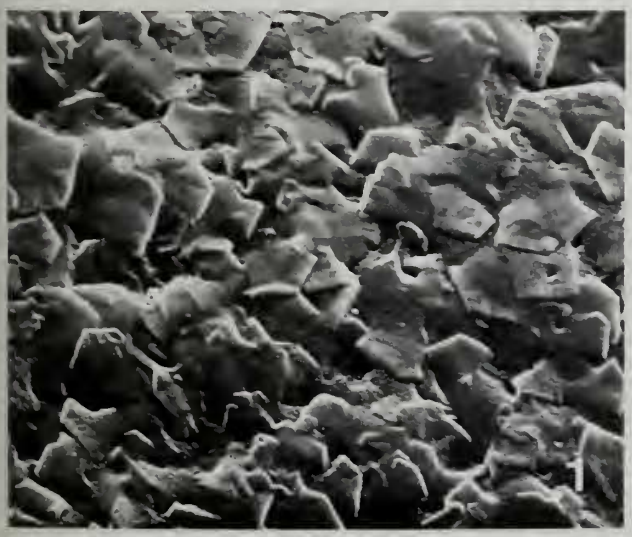
By the 7th day of forced adduction, live clams showed considerable disruption of internal shell microstructure. In the central portion of the internal shell, there was little organization of calcarcous structures. Instead, a reticulated microstructural pattern of apparently high organic material was present (Figure 7). This obscured any organized microstructure that might have otherwise been present beneath the eroded surface.

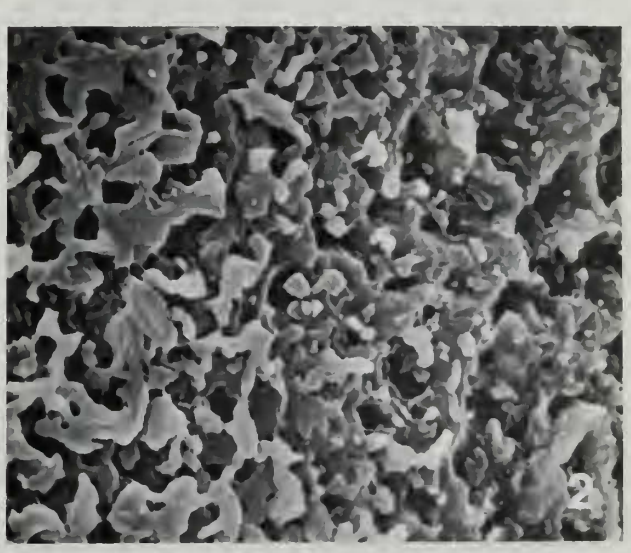
In specimens treated for 30 sec with a 50% solution of hydrochloric acid, a pattern of microstructures similar to the reticulate pattern noted above was obtained (Figure 8). A reticulate matrix high in organic material remained after apparent dissolution of calcareous features. Following treatment with an organic solvent (*i.e.*, sodium hypochlorite), a smooth surface remained.

Field Controls

Clams brought into the laboratory alive but apparently in an "unhealthy" condition (*i.e.*, weak adduction response, sluggish foot activity) showed pure white internal shell coloration and typically an internal shell microstructure that resembled crossed-acicular (or an eroded form of complex crossed-lamellar).

All clams examined directly from the field in healthy condition (*i.e.*, rapid adduction response, active pedal movement, rapid burrowing) showed internal shells with purple highlights and a typical complex crossed-lamellar microstructure internal to the pallial line (see Prezant & Tan Tiu, 1985).

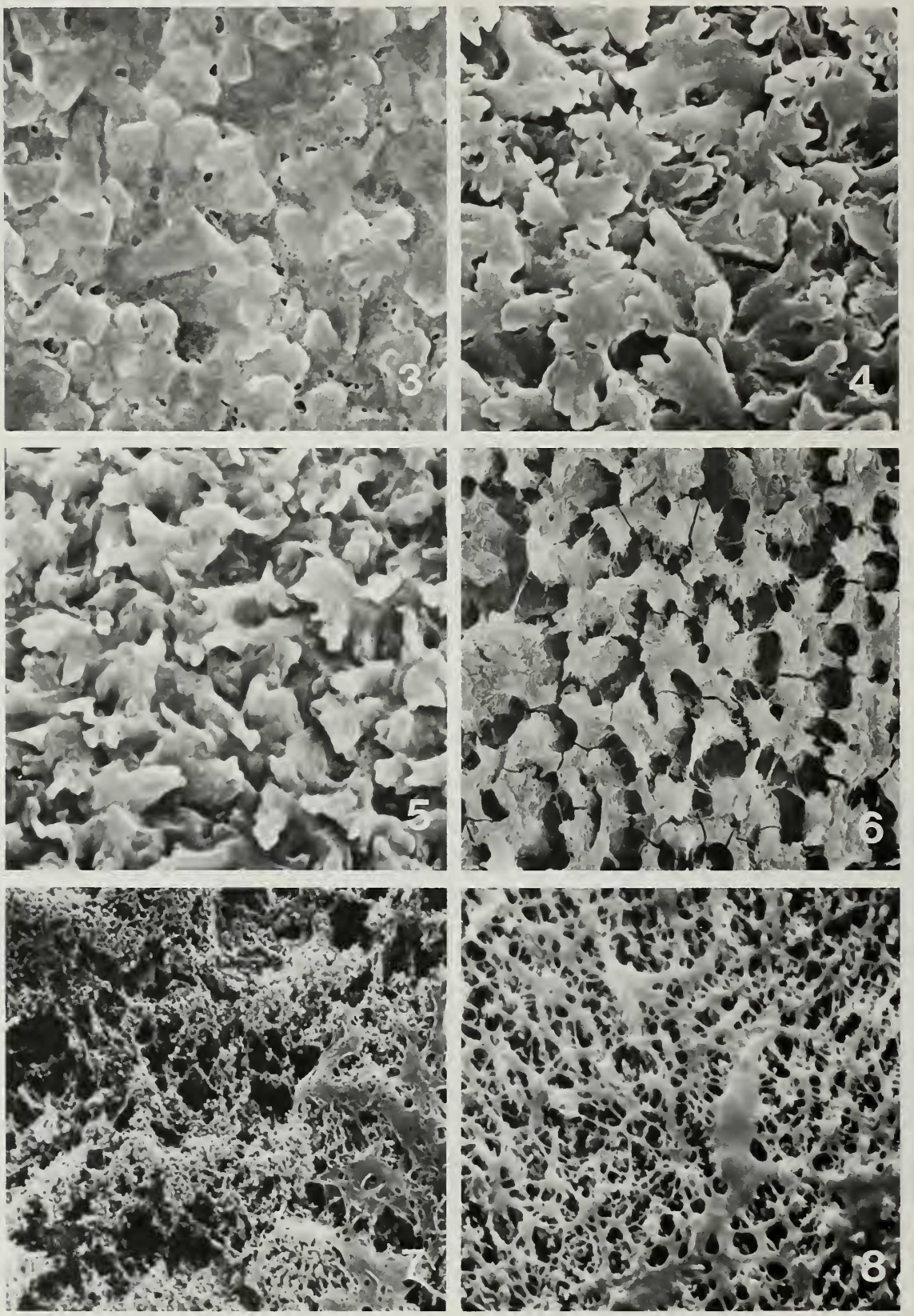




Explanation of Figures 1 and 2

Figure 1. Surficial view of complex crossed-lamellar lath tips in "normal" shell of *Corbicula fluminea*. [All photographs are scanning electron micrographs.] Horizontal field width = 2 μm.

Figure 2. "Crossed-acicular" microstructure induced by subjecting small *C. fluminea* specimens to warm, high organic waters followed by a lethal rise in aquarium temperatures. Horizontal field width = 2 μm.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DISCUSSION

The surficial microstructures of complex crossed-lamellar layers are often irregularly arranged. TAYLOR *et al.* (1969) have noted several bivalve species that have surficial complex crossed-lamellar microstructures that have a "confused surface pattern." In these the second-order lamels "vary in attitude" creating third-order lamels with an apparent random orientation. The physiological status of specimens examined by TAYLOR *et al.* (1969) was not documented. A discussion of variation in complex crossed-lamellar structures can be found in TAYLOR *et al.* (1969) and CARTER (1980a).

CARTER (1980b) defines crossed-acicular microstructures as similar to crossed-lamellar "except that each aggregation consists of only a few parallel elongate subunits." The conversion of crossed-lamellar internal shell microstructures to "crossed-acicular" microstructures (in essence nondiscernible from some erosive patterns shown by CRENSHAW [1980]) raises interesting questions concerning nomenclatural validity of specific crossed-lamellar microstructures. In *Corbicula fluminea* it appears that crossed-acicular microstructures are an aberrant form of complex crossed-lamellar microstructures with the irregularity of laths being either (1) a modification of the typical shell microstructure, or (2) a new type of deposit produced under specific conditions, or (3) the result of dissolution phenomena. It is difficult to assume that the crossed-acicular-like deposits are the result of usual erosive events, as no erosive pitting was evident nor was this type of microstructure consistently found in bivalves subjected to forced adduction. Instead, a more erosive type of dissolution surface was present inside the valves of specimens that were forced to remain closed for long periods. In specimens that were clamped for up to 7 days, the internal shell microstructure bore little resemblance to the crossed-acicular pattern. The build-up of acids in the extrapallial fluid during adduction seemingly impinges first upon the calcified microstructures and leaves an organic residue behind. This is indeed what is typically seen in long-term adducted specimens we examined and is considered predictable. (For example, CURREY [1988] briefly discusses the erosive resistance of "conchiolin," at least in deterring some drilling predators; see also LEWY & SAMTLEBEN [1979].) In shorter-term studies (*i.e.*, 24 h) with *Corbicula*, we found microstructures that at least broadly resembled crossed-acicular patterns.

TAN TIU (1987) observed that valves of recently sacrificed *Corbicula fluminea* placed in the Leaf River and Eddy Lake of Mississippi for at least 3 months also had white internal shell coloration but lacked crossed-acicular patterns. Shell microstructures dominating were primarily elongate structures resembling remnants of dissolution in non-living systems. This indicates that crossed-acicular patterns found in *C. fluminea* from empty shells collected along the shore could have been formed prior to the death of the animal. Moreover, in longer-term thermal and trophic experiments (Prezant & Tan Tiu, unpublished data), not all internal shell surfaces of dead clams were white and no crossed-acicular microstructures were observed. TAN TIU (1987) did observe crossed-acicular microstructures in clams collected live from the Leaf River but only rarely (*i.e.*, 1 of 30 in September 1985, 9 of 28 in October 1985, and 2 of 30 in March 1986). These conflicting data make our present results equivocal and we have yet to discern the exact cause of the modified shell.

CRENSHAW & NEFF (1969) and CRENSHAW (1980) demonstrated distinct differences in interior shell surfaces proximal to the pallial line of *Mercenaria mercenaria* (Linnaeus) following emergence from water for 3 h. They found that following emersion, crystals were "poorly organized with large voids on the inner surface" while those outside the pallial line retained "sharp edges" and organization and filled "available space." CRENSHAW (1980) noted, however, that microstructures inside and outside the pallial line in *M. mercenaria* are normally different (as they are in *Corbicula fluminea*) with the outer shell dominated by crossed-lamellar and prismatic, and the inner shell consisting of complex crossed-lamellar and/or homogeneous microstructures. CRENSHAW (1980) characterized the shell found inside the pallial line after aerial exposure as characteristic of erosive dissolution that occurs during anaerobiosis with the build-up of acids in the extrapallial fluid. This same argument can be made for *C. fluminea* if we could be sure that anaerobiosis was the stimulus. In our experiments, stressed clams usually remained slightly abducted and this could preclude the build-

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Explanation of Figures 3 to 8

Figure 3. During 24 h of forced adduction, bivalves (*Corbicula fluminea*) frequently showed increased porosity of organic layers surrounding lamel tips. Horizontal field width = 2 μm.

Figure 4. During 24-h forced adduction studies, lamel tips were often disrupted and showed dendritic qualities. Horizontal field width = 2 μm.

Figure 5. Final phases in 24-h adduction dissolution studies revealed microstructures that had complete disruption of organized lamels and microstructurally resembled a stage intermediate between crossed-acicular patterns and complete dissolution patterns (see Figures 2, 6). Horizontal field width = 2 μm.

Figure 6. Complete disruption of organized lamels results after 24 h of forced adduction. Horizontal field width = 10 μm.

Figure 7. After 7 days of forced adduction, bivalves showed a reticulate pattern of apparently organic material in the central portion of the shell interior. Horizontal field width = 2 μm.

Figure 8. Central interior of the shell of a specimen of *C. fluminea* treated for 30 sec with 50% hydrochloric acid. The resulting reticulate pattern resembles the pattern seen in Figure 7. Horizontal field width = 2 μm.

up of erosive acids in the extrapallial space. In addition, CRENSHAW & NEFF (1969) found that dissolution probably was a rapid result of anaerobiosis and thus erosion should begin quickly. Specimens of *C. fluminea* stressed by forced adduction showed similar signs of modified internal shell as in clams that were experimentally stressed by heat and organic water load over longer periods of time (*i.e.,* 24 h force adducted vs. several days in warm, high organic waters) (latter from unpublished data, Prezant, Tan Tiu and Chalermwat). Micrographs shown by CRENSHAW (1980) of complex crossed-lamellar shell of *Mercenaria mercenaria* that had undergone dissolution are reminiscent of modified *Corbicula* shell resembling crossed-acicular. In *Mercenaria,* however, the pattern shows fewer elongate units. If any of our specimens that showed crossed-acicular patterns were physiologically stressed, the appearance of a porous microstructure (showing gaps in organic deposits) could be envisioned as being energy efficient. The erosion of a complex crossed-lamellar microstructure in an "inactive" extrapallial fluid or conchiolin base can also be envisioned as energy saving. These patterns of shell deposition or shell dissolution are distinct from the eroded internal shell seen after 7 days of forced adduction. It is possible that the two forms are a continuum of each other (*i.e.,* phases of progressive dissolution). We suggest that the internal shell surface seen after 7 days of adduction represents strongly eroded shell (compare with HCl-treated shell) that has had most surficial calcareous material dissolved. The crossed-acicular-like microstructure either is a "milder" type of erosive pattern (induced by lower stress without the build up of high concentrations of erosive extrapallial fluid acids) or a metabolically inexpensive form of shell deposition (low in organics).

It is possible that the organism produces the modified complex crossed-lamellar structures as a result of a shift in body energetics. Thus far there is no definitive work that has discerned the energy costs of producing molluscan shell, in particular the partitioned costs of producing the calcium carbonate portion versus the organic matrix. CURREY (1988) reviews the little that is known of this subject and briefly discusses the work of PALMER (1983) that indicates a higher energy cost for producing the organic portion of shell. Under stressed conditions mollusks could shift energy reserves toward life-sustaining functions, which may not include shell production in post-larval animals. Thus, under stress the bivalve could terminate normal shunting of incipient shell materials through the mantle, thereby producing a modified extrapallial fluid that could be conducive to modified shell production. Chalky deposits seen in ostreid bivalves are reminiscent of this situation. CARRIKER *et al.* (1980) and STENZEL (1971) point out that chalky shell deposits are composed of less calcareous material than a comparable shell volume of typical foliated structure. The supposition is that chalky deposits are more economical to produce than more compact, regular foliated shell. If shell organic matrix (*i.e.,* conchiolin) is energetically more expensive to produce in corbiculid and other bivalves, perhaps we need to look towards temporal modifications of the type of organic material being produced under given circumstances to account for differences in shell microstructure.

The origin of the modified "complex crossed-acicular" shell microstructure we found using stressed *Corbicula fluminea* remains unclear. We must exercise extreme care, however, in separating a crossed-acicular microstructure from the erosive remains of a crossed-lamellar microstructure. We might speculate here that stress in some instances will induce internal shell erosion; in others stress could produce replacement deposition in the form of an energetically cheaper shell microstructure.

Very little is known about the influence that environment has on biomineralization or crystallization in mollusk shell. Since different environmental regimes produce different growth rates (MALONE & DODD, 1967) it is likely that these variations will be reflected in shell microstructure. TAN TIU (1988), for instance, has found significant temporal (seasonal) changes in shell microstructure within the bivalve *Polymesoda caroliniana* (Bosc). Subtle variations in shell microstructure in *Corbicula fluminea* can be induced by variation in microhabitat in conjunction with the bivalve's physiological condition.

Variations in dissolution and deposition patterns lead to a wide variety of microstructural forms. To extrapolate our present data on internal shell color and microstructure to help decipher the taxonomic quagmire developed in the debate over the number of species of North American *Corbicula* would be premature. This is particularly true in light of our equivocal results based on the present report and unpublished data. The debate continues but we argue strongly to limit our discussions to valid taxonomic characters and move away from shell color and subtle shell morphometrics.

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